Final

July 3, 2014

Derivation of an Extrapolated Short-term Inhalation Screening Level for 4-Methylcyclohexanemethanol (MCHM – CAS# 34885-03-5)

U.S. Environmental Protection Agency (EPA)
Office of Research and Development (ORD)
National Center for Environmental Assessment (NCEA)
Superfund Health Risk Technical Support Center (STSC)

Background:

Currently, there are no available repeated-dose inhalation toxicity studies on 4-methylcyclohexanemethanol (MCHM) in humans or animals. There is a single short-term repeated-dose oral study conducted in rats. In this study, male and female CD(SD)BR rats (5/sex/dose group) were administered MCHM (purity >96%) via gavage at doses of 0, 25, 100, and 400 mg/kg-day, 5 days per week, for 28 days (Eastman Kodak Company, 1990). The lowest-observed-adverse-effect level (LOAEL) for this study is 400 mg/kg-day based on erythropoietic, kidney (increased tubular degeneration), and liver (increased weight and inflammation) effects. The associated no-observed-adverse-effect level (NOAEL) is 100 mg/kg-day. Detailed descriptions of all available toxicity studies for pure MCHM and crude MCHM can be found on the National Library of Medicine's Hazardous Substances Data Bank (HSDB, 2014).

There are no available toxicity values for MCHM from the U.S. EPA's Office of Solid Waste and Emergency Response (OSWER) recognized Tier 1–3 sources or the International Toxicity Estimates for Risk (ITER) database. Recently, the Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry (CDC/ATSDR) established a short-term health advisory for MCHM in drinking water in and around Charleston, West Virginia after approximately 7500 gallons of the chemical was spilled into the Elk River (CDC/ATSDR, 2014). CDC/ATSDR's short-term drinking water advisory for MCHM of 1 part per million (ppm) is based on the NOAEL of 100 mg/kg-day from the aforementioned 28-day gavage study in rats. The drinking water (DW) advisory was derived to be protective of children as follows:

$$\text{DW Advisory} = (\text{NOAEL} \times \text{BW}) \div (\text{UF}_C \times \text{Intake})$$

$$\text{DW Advisory} = (100\ \text{mg/kg-day} \times 10\ \text{kg}) \div (1000 \times 1\ \text{L/day})$$

$$\text{DW Advisory} = 1\ \text{mg/L (or 1 ppm)}$$

Where:

- NOAEL = No-observed-adverse-effect level from 28-day gavage study in rats
- BW = Body weight of a child (10 kg)
- UF_C = Composite uncertainty factor ($UF_A \times UF_H \times UF_D$) applied to account for (1) uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability; $UF_A = 10$), (2) human-to-human variability in susceptibility and consideration of possible effects on vulnerable populations, including pregnant women and children ($UF_H = 10$), and (3) the limited availability of data and database deficiencies – e.g., lack of reproductive and developmental toxicity studies ($UF_D = 10$).
- Intake = Estimated drinking water intake of a 10 kg child (1 L/day).

Derivation of an Extrapolated Short-term Inhalation Screening Level:

Assessing the potential public health impacts of inhalation exposures to MCHM is difficult due to the lack of any available inhalation toxicity data. However, using CDC/ATSDR's short-term DW advisory for MCHM of 1 mg/L (or 1 ppm), it is feasible to conduct an oral route-to-inhalation route extrapolation in an attempt to estimate a short-term inhalation screening level. The methodologies and derivations presented herein were reviewed by scientists within the U.S. EPA and independently peer-reviewed by scientists at the CDC/ATSDR, National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program (NTP), and National Library of Medicine (NLM).

Applying the 1 L/day estimated intake from drinking water of a 10 kg child used to derive CDC/ATSDR's DW advisory of 1 mg/L (or 1 ppm), this DW advisory value can be expressed in mg/kg-day as follows:

DW advisory (mg/L) × Intake ÷ BW = DW advisory (mg/kg-day)

1 mg/L × 1 L/day ÷ 10 kg = 0.1 mg/kg-day

In the absence of any available pharmacokinetic information on MCHM, route-to-route extrapolation of CDC/ATSDR's DW advisory (derived to be protective of children) to an extrapolated short-term inhalation screening level can then be derived as follows:

Extrapolated Short-term Inhalation Screening Level = DW advisory × (BW ÷ IR)

= 0.1 mg/kg-day × (10 kg ÷ 17.3 m^3/day)

= 0.06 mg/m^3

Where:

- BW = Body weight of a child (10 kg)
- IR = Inhalation rate for a child 1 to < 2 years old (U.S. EPA, 2011; calculated using the recommended mean light intensity short-term exposure value for inhalation of 1.2×10^{-2} m^3/min × 60 min/hr × 24 hr/day).

Extrapolated Short-term Inhalation Screening Level converted to ppm (in air):

Air Conc (ppm) = Air Conc (mg/mg^3) ÷ Molecular Weight of MCHM × 24.45

Air Conc (ppm) = 0.06 mg/m^3 ÷ 128.21 × 24.45

Air Conc (ppm) **= 0.01 ppm (in air)**

Important Limitations and Uncertainties:

It is important to note that the information presented herein provides an extrapolated short-term inhalation screening level for MCHM that is not intended to provide conclusive estimates of actual risk from inhalation exposures of MCHM, or generate remediation/cleanup goals. Additionally,

cognizance of the several limitations and uncertainties associated with the derivation of this extrapolated short-term inhalation screening level is essential.

First, an oral route-to-inhalation route dosimetric extrapolation does not account for efficiency of respiratory tract deposition and distribution of a chemical, biological and physicochemical factors, and other potential inhalation exposure scenarios (e.g., continuous versus intermittent exposure) that may affect uptake and clearance. Therefore, this simple extrapolation method implicitly assumes that the route of exposure is unrelated to the delivered target organ dose, which is not supported by the fundamental tenets of dosimetry or toxicokinetics. Additionally, if MCHM undergoes a first-pass effect, the blood concentration of the parent compound from oral exposure may be lower than the blood concentration following inhalation exposure at an equivalent concentration.

Second, although CDC/ATSDR used the best available repeated-dose oral toxicity study conducted on MCHM in animals to derive its DW advisory, the test compound used in the study was pure MCHM which is only comprised of the major constituent of crude MCHM (the actual compound that was spilled, composed of a mixture of synthetic chemicals).

Third, although the database lacks inhalation studies on MCHM, a potential for pulmonary portal-of-entry effects may exist as indicated by the observation that MCHM is a dermal and ocular irritant in animal studies (HSDB, 2014).

Finally, due to the short-term-duration of the oral study used to derive CDC/ATSDR's DW advisory and the uncertainty associated with applying this value to longer duration oral exposures, the extrapolated short-term inhalation screening level is not applicable to longer-term/chronic-duration inhalation exposures.

References:

CDC/ATSDR (Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry). (2014). Summary Report of Short term Screening Level Calculation and Analysis of Available Animal Studies for MCHM.

Eastman Kodak Company. (1990). Four-week oral toxicity study of 4-methylcyclohexane methanol in the rat.

HSDB (Hazardous Substances Data Bank). (2014). 4-Methylcyclohexanemethanol. Bethesda, MD: National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@DOCNO+8182

U.S. EPA (U.S. Environmental Protection Agency). (2011). Exposure Factors Handbook 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F.

1/20/2014

Summary Report of Short-term Screening Level Calculation and Analysis of Available Animal Studies for MCHM

This summary report explains how the Centers for Disease Control and Prevention (CDC) used generally accepted scientific methods[1] to establish a short-term health advisory for drinking water in and around Charleston, West Virginia immediately after a chemical spill in the Elk River. The summary report also shows how CDC used animal studies—once they became available—to validate the initial short-term screening level of 1 part per million (ppm) calculated during the early stages of the emergency response.

Background

On January 9, 2014, approximately 7500 gallons of an industrial chemical, 4-Methylcyclohexanemethanol (MCHM – CAS# 34885-03-5), spilled into the Elk River just upstream from the Kanawha County municipal water intake in Charleston, West Virginia. This municipal water system serves nearly 300,000 people whose water was affected by the chemical spill. Due to the uncertainty over the chemical levels in the water supply, the Office of the Governor issued a "Do Not Use" order at 6:00 pm on January 9, 2014. Later that evening, the West Virginia Department of Health and Human Resources contacted CDC about the release and requested assistance to review water sampling data and provide a drinking water screening level for MCHM. In response to this urgent situation, a screening level of 1ppm was recommended. Based on the information available, a level of 1ppm or below is not likely to be associated with any adverse health effects.

Summary

The Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry (CDC/ATSDR) used the most relevant available information to provide a scientifically supported recommendation for the protection of public health. Initial actions in the Charleston area were based upon limited available information and resulted in a decision to issue a short-term public health alert regarding all municipal water use in the Kanawha water distribution area.

The initial short-term screening level that CDC/ATSDR recommended for the Elk River spill followed common practices for the interpretation of toxicological information for public health purposes (see Methodology section below). Often such information is incomplete; this was the case for MCHM in this incident. CDC/ATSDR analyzed additional information, once it became available, to verify the initial short-term screening level and health advisory.

Data Review

On the evening of Thursday, January 9, 2014 emergency response staff from CDC/ATSDR, in cooperation with local and state health authorities, recommended an interim urgent short-term screening level based upon information available at the time. The short-term

screening level was used to issue a warning to users of the local water district to avoid all contact with municipal water.

Since CDC/ATSDR recommended the emergency short-term screening level, state and federal officials have acquired several additional proprietary studies on the toxicology of MCHM. When these studies became available, the U.S. Department of Health & Human Services convened a Federal expert workgroup including scientists from the National Institute of Environmental Health Sciences and National Toxicology Program, the National Library of Medicine, the Environmental Protection Agency, and CDC/ATSDR to review the available animal studies and the methodology for the short-term screening level calculation. This workgroup concurred that the 1 ppm short-term screening level was appropriate.

The studies of pure MCHM and crude MCHM are summarized below. For a more detailed description of these studies, please see the National Library of Medicine's Hazardous Substances Data Bank: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@DOCNO+8182.

1) Crude MCHM. Ames test for mutagenic potential. This test used multiple *Salmonella typhimurium* strains and one *E. coli* strain, with six doses, and with and without "S9 mix" to study the impact of possible metabolism of activity. No increase in revertants was noted. A repeat study confirmed these results.

2) Crude MCHM. Two-week daily dermal application. Male and female rats were dosed at 0, 100, 500, and 2000 mg/kg/day, 6 hours/day for 13 consecutive days. There was dermal irritation at all treatment levels and thus no No Observed Effect Level (NOEL) was identified. Because of the absence of significant histopathology and serum clinical chemistry changes, 2000 mg/kg was considered as the NOEL for systemic toxicity. One focus was to look at hematuria as a possible toxic effect seen in an earlier acute dermal study.

3) Crude MCHM acute single dose dermal. A single dose of 2000 mg/kg was applied to male and female rats, with a 14-day observation period. Dermal irritation was observed, and the dermal LD50 was greater than 2000 mg/kg.

4) Crude MCHM acute single dose oral. Male and female rats were dosed with 500, 1000, and 2000 mg/kg, followed by a 14-day observation period. The acute LD50 was calculated as 933 mg/kg for males and 707 mg/kg for females.

5) Pure MCHM 28 day daily oral. Rats received 0, 25, 100, and 400 mg/kg/day, 5 days a week, for 4 weeks. In this study, the administration of 400 mg/kg/day for 4 weeks was associated with erythropoietic, kidney, and liver effects, including increased liver weight, inflammation, and kidney tubular degeneration. The authors set a NOEL at 100 mg/kg/day. ***CDC used this study to establish the short-term health advisory for the MCHM spill in the Elk River.***

6) Crude MCHM acute single dose oral (repeat of earlier study). This is a study of a single oral dose to female rats (i.e., to look at hematuria as a possible toxic effect as seen in another study). The LD50 was calculated to be 500 mg/kg.

7) Pure MCHM acute battery:

a) Acute single dose oral toxicity. Male and female rats were dosed at 625, 1250, and 2500 mg/kg. The estimated LD50 was 1768 mg/kg in males and 884 mg/kg in females.

b) Acute single dose dermal exposure. Male and female rats were dosed at 2, 6, and 20 ml/kg. MCHM was irritating to skin at as low as the 2 ml/kg dose, but only in females at this dose.

c) Acute single exposure dermal irritation. Guinea pigs were dosed at 0.5 ml on the abdomen and covered with occlusive wrapping for 24 hours. They were observed for 48 hours after the wrap was removed. MCHM exposure led to strong skin irritation.

d) Acute toxicity, repeated application to skin. The backs of guinea pigs were exposed to 9 doses of 0.5 ml of MCHM "drop on" over 11 days. There was "... exacerbation of the irritant response with (multiple) application..."

e) Acute toxicity, evaluation of skin sensitizing potential. Male guinea pig footpads were exposed to 0.05 ml of a 1.0% solution MCHM in adjuvant (FCA) for induction. No sensitization was observed after a challenge application, and MCHM was concluded to have "a low potential to cause human sensitization."

f) Acute toxicity, eye irritation. One dose of 0.1 ml of MCHM was placed onto the eyes of rabbits, followed by washing or no washing. The washed eyes showed slight irritation, and the unwashed eyes showed moderate irritation. MCHM was concluded to be a "moderate irritant."

Together, these studies provide a much-improved (but still incomplete) understanding of MCHM's toxicology profile. In particular, one of the studies, the 4-week rat study (study 5 above), provides a NOEL in rats. This NOEL, established by the authors of the study, is 100 mg/kg/day. The 4-week NOEL represents a more scientifically sound study and point of departure for establishing a short-term health advisory for MCHM.

Methodology

CDC/ATSDR used the following methodology to establish a short-term screening level of 1 part per million (ppm) for the MCHM spill in the Elk River:

$$DW\ Advisory \leq \frac{NOEL \times BW}{UF \times Intake}$$

Where:

- DW Advisory = Short-term Drinking Water Advisory
- NOEL = No Observed Effect Level in the experimental species (100 mg/kg/day)
- BW = Body weight of a child (10 kg)
- UF = Uncertainty factors that address differences between animals and humans (10X), address differences accounting for sensitive humans (10x), and account for weaknesses in the toxicological database (10X). For example, there are no developmental, neonatal, or transplacental studies available.
- Intake = The estimated intake from drinking water of a 10 kg child (1 liter/day).

NOEL (mg/kg/d)	BW (kg)	UF (unitless)	Intake (L/day)	Short-term DW Advisory (mg/L or ppm)
100	10	1000	1	1

[1] Donohue, J.M. and Libscomb, J.C. Health advisory values for drinking water contaminants and the methodology for determining acute exposure values. *Sci Tot Env.* 288 (2002) 43-49.

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TX-89-296

FOUR-WEEK ORAL TOXICITY STUDY OF 4-METHYLCYCLOHEXANE METHANOL IN THE RAT

HAEL NO. 89-0081 ACC. NO. 907670

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261930L
TX-89-296

FOUR-WEEK ORAL TOXICITY STUDY OF 4-METHYLCYCLOHEXANE METHANOL IN THE RAT

HAEL NO. 89-0081 ACC. NO. 907670

Abstract

Groups of two male and two female rats were given doses of 200, 400, or 800 mg/kg/day of 4-methylcyclohexane methanol in corn oil for five days as part of a probe study conducted to establish dose levels for the four-week toxicity study. Rats dosed with 800 mg/kg showed signs of narcosis resulting in decreased activity levels (one male and two females) and ataxia (one female). One of the female rats was subsequently euthanatized. One of the 400 mg/kg/day females had decreased activity on Days 2 and 3 of the study. The remaining animals did not exhibit clinical abnormalities related to exposure to the test article. Dose levels of 0, 25, 100, and 400 mg/kg/day were chosen for the four-week study based on these results.

In the four-week study, the test article was administered five days per week by gavage in corn oil to groups of five male and five female rats. No mortality was observed during this study. Minimal reductions in body weight growth were present for both male and female rats given the high-dose of the test article. These differences were not statistically significant. At lower dose levels, no consistent effect was noted. Males given the lower doses weighed slightly less than their control group while females weighed slightly more. Feed consumption was unaffected by administration of the test material.

Sialorrhea after dose administration occurred frequently in the 400 mg/kg male and female dose groups from Days 14 to 28. Transient depression of activity occurred in one 400 mg/kg female animal on Day 3 of the study. These were the only two treatment-related clinical observations noted.

Hematologic changes indicative of minimal anemia were observed in the 400 mg/kg female group. These changes included a significantly decreased mean red blood cell count relative to the control group, and lower mean values for hemoglobin and hematocrit. In the absence of evidence of increased red blood cell destruction or turnover, these results suggest an interference with erythropoiesis rather than a direct effect on circulating red blood cells.

Male and female rats from the 400 mg/kg dose group had significant increases in mean serum creatinine levels relative to their respective control groups, although the differences were not clearly of biological significance as urea nitrogen levels were not similarly increased. Microscopic examination of the kidneys of the 400 mg/kg animals revealed scattered areas of degeneration of the proximal convoluted tubules in 2 out of 5 animals of each sex. While mean relative kidney weights of all male treatment groups were statistically significantly heavier than their control group, the differences did not fit a dose-related pattern.

Abstract (Cont.)

Male rats from the 400 mg/kg dose group had significantly higher mean serum aspartate transaminase (AST) and sorbitol dehydrogenase (SDH) levels when compared to their control group. While the high-dose female group did not exhibit similar increases, one of the high-dose females did have an elevated SDH level and the mean relative liver weight for the female high-dose group was statistically significantly increased at the 400 mg/kg dose level. Microscopic examination of the livers from the 400 mg/kg animals of both sexes revealed increased severity and wider distribution of chronic focal inflammation in three males and two females when they were compared to their control groups.

In summary, administration of 400 mg/kg/day of the test article for four weeks was associated with erythropoietic, kidney, and liver effects. None of the effects were indicative of more than minor toxicity, and all were most likely reversible. The no-observed-effect level for this subacute toxicity study was 100 mg/kg/day.

261930L
TX-89-296

FOUR-WEEK ORAL TOXICITY STUDY OF 4-METHYLCYCLOHEXANE METHANOL IN THE RAT

PURPOSE

The purpose of this study was to evaluate the subacute effects of 4-methylcyclohexane methanol when given to rats orally for four weeks.

TESTING FACILITY

Toxicological Sciences Laboratory
Health and Environment Laboratories (HAEL)
B-320 Kodak Park
Eastman Kodak Company
Rochester, NY 14652-3615

TEST ARTICLE CHARACTERIZATION

Chemical Name: 4-methylcyclohexane methanol
HAEL No.: 89-0081
EK Acc. No.: 907670
CAS No.: 34885-03-5
SRID OR Lot No.: X20511-3-3
Experiment No.: 890081G1

PURITY, STABILITY, and CONCENTRATION ANALYSES

The purity of the test article was 97.3% prior to study initiation and 96.6% at study termination, when analyzed by gas chromatography.

Stability of the test article in corn oil was determined by repeated analysis of 1.0 and 20.0% solutions of the test article using gas chromatography on Day 0 and 1, 4, and 9 days after test solution preparation. Concentrations of the test article were 1.0±0%, and 20.0±0% (mean ± SD) prior to storage and 1.0±0%, and 20.0±0% after 9 days of storage, indicating the material was stable in corn oil for at least 9 days.

The concentration of the test article in each batch of test solution was determined prior to use by gas chromatography. The mean concentrations of the test article were 0.6±0.04%, 2.0±0.2%, and 7.8±0.5% (mean ± SD) compared to target concentrations of 0.5, 2.0, and 8.0%.

All analyses were performed by the Chemical Quality Services Division, KP.

TEST PROCEDURE

This study was conducted by methods comparable to OECD GUIDELINES for TESTING of CHEMICALS TG-407, Repeated Dose Oral Toxicity - Rodent: 28-day or 14-day study, and Annex V B.7.

TEST SUBSTANCE EXPOSURE

Rats were given 25, 100, or 400 mg/kg of the test material in corn oil for 21 doses over 29 days. Doses were determined based upon the results of a one week probe study. Doses were given five days per week including holidays. Controls received daily doses of corn oil in volumes equal on a mL/kg body weight basis to those administered to the test groups.

ANIMALS

Five male and five female rats (CD®(SD)BR) from Charles River Laboratories, Kingston, NY, were randomly assigned to each test group. Animals were isolated prior to testing. At the start of the study, rats were approximately 45 (males) or 50 (females) days old and weighed 214±6 g (males) or 173±6 g (females) (mean ± SD). Rats were chosen for this study because they are a common representative species for toxicity studies.

HOUSING

Rats were housed in groups of five segregated by sex. The study was conducted in the vivarium area of Building 320. The study room was maintained at 68-74 °F and 50-61% relative humidity. A photoperiod of 12 hours from 6 a.m. to 6 p.m. was maintained. No other study was housed in the same room as this study. Cages and racks were washed once a week. Absorbent paper under the cages was changed daily.

FEED AND WATER

Agway® Prolab™ Animal Diet (RMH 3200), certified ground chow was fed ad libitum. Feed containers were cleaned weekly. Feed containers were refilled at least once a week. Water was supplied ad libitum through an automatic watering system. The source of the water was the Monroe County Water Authority. No known contaminants in feed or water were expected which would interfere with the outcome of this study.

IDENTIFICATION

All rats were identified by uniquely numbered metal ear tags and ear punches.

RANDOMIZATION

All culling and randomization were done by computer-generated lists using the Automated Animal Toxicology System.

BODY AND FEED WEIGHT DETERMINATIONS

Body weights were collected on Days 0, 4, 7, 14, 21, and 28. Feed consumption was determined on Days 4, 7, 11, 14, 18, 21, 25, and 28.

CLINICAL OBSERVATIONS

Every workday morning each rat was removed from its cage and examined by a trained technician. Immediately after dosing and again in the afternoon, cageside observations were conducted. Cageside observations included, but were not limited to, examination of the hair, skin, eyes, motor activity, feces, and urine. Animals were checked for mortality on weekends.

HEMATOLOGY AND CLINICAL CHEMISTRY EXAMINATIONS

At the time of necropsy, blood was collected from the posterior vena cava while the rats were under CO_2 anesthesia. All assays were conducted by the Animal Clinical Analysis Group, HAEL. Hematology tests included: hemoglobin concentration, hematocrit, red blood cell count, white blood cell count, differential white blood cell count, platelet count, red blood cell indices, and examination of the blood smears for cellular morphology. Clinical chemistry tests included: aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, creatinine, urea nitrogen, and glucose.

NECROPSY

Rats were fasted overnight, anesthetized with CO_2, and exsanguinated by severing the posterior vena cava after collecting blood for analysis. Necropsies were conducted according to pathology SOP No. TP 180. The liver, kidneys, adrenal glands, testes, spleen, and thymus were weighed. Paired organs were weighed together. Organ/body weight ratios were calculated. The following organs were fixed in 10% buffered formalin: trachea, lungs, heart, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, pancreas, liver, salivary glands, kidneys, urinary bladder, pituitary gland, adrenal glands, thyroid glands, parathyroid glands, thymus, spleen, mesenteric lymph nodes, bone marrow (femoral), brain, testes, epididymides, male accessory sex glands, ovaries, vagina, uterus, Fallopian tubes, and gross lesions. All tissues were examined microscopically from the control and high-dose groups and gross lesions and target organs were examined from other groups.

STATISTICAL PROCEDURES

Mean values were calculated for body weight, feed consumption, organ weights, hematology, and clinical chemistries. All mean data, except feed consumption, were evaluated using the following computer-generated statistical tests: Bartlett's test ($p \leq 0.01$), one-way analysis of variance (ANOVA) ($p \leq 0.05$), and Duncan's multiple range test ($p \leq 0.05$) to indicate statistical significance. Feed consumption was not analyzed statistically because the animals were group housed.

DATA STORAGE

The final report, tissues, paraffin blocks, slides, data sheets, and all nonperishable raw data were stored in the HAEL archives.

GLP STATEMENT

This study was conducted according to Good Laboratory Practice for Nonclinical Laboratory Studies as promulgated by the Food and Drug Administration, 21 CFR Part 58, December 22, 1978 amended September 4, 1987; Environmental Protection Agency Good Laboratory Practice Standard 40 CFR Part 792, November 29, 1983; and Annex 2 of the OECD Guidelines for Testing of Chemicals (C(81)30 (Final)) as required by Council Directive 87/18/EEC of December 18, 1986.

PROJECT PARTICIPANTS

Study Director	G. J. Hankinson, D.V.M., M.S.
Study Technician	T. S. Hill, A.A.S.
Necropsy Pathologist	M. S. Vlaovic, D.V.M., Ph.D.
Histopathologist	M. S. Vlaovic, D.V.M., Ph.D.
Laboratory Animal Medicine	G. J. Hankinson, D.V.M., M.S.
Hematologist/Clinical Chemist	R. E. Emmons, B.S.
Analytical Chemist	K. A. Robillard, Ph.D.
Report Author	R. S. Hosenfeld, A.A.S.

STUDY DATES

Study Initiation Date	July 14, 1989
Experimental Start Date	July 17, 1989
Experimental Termination Date	November 7, 1989

PROTOCOL AND SOP DEVIATIONS

There were no protocol or standard operating procedure (SOP) deviations.

RESULTS

PROBE STUDY

A probe study was conducted to assist in dose level selection for the four-week study. In the probe study, groups of two male and two female rats were administered 0, 200, 400, or 800 mg/kg/day of the test article in corn oil for a period of five days. Abnormal clinical signs observed in animals receiving doses of 800 mg/kg/day included ataxia and decreased activity in both female rats and decreased activity in one of the two male rats. One of the 800 mg/kg female rats developed signs of severe central nervous system depression. After the fourth dose, this animal became hypothermic and moribund, and was euthanatized. The surviving 800 mg/kg female and one of the 800 mg/kg males lost weight during the study. No abnormal clinical signs were noted for the 400 mg/kg male group. Decreased activity was noted for one of the 400 mg/kg females during afternoon observation periods on Days 2 and 3. This animal appeared normal at all observation times on Day 4. No abnormal clinical signs were seen for the other 400 mg/kg female. No abnormal clinical signs were seen in animals given doses of 200 mg/kg. All animals administered doses of 200 or 400 mg/kg/day gained weight during the study. Based on the results of the probe study, dose levels of 0, 25, 100, and 400 mg/kg/day were chosen for the four-week study.

MORTALITY

No mortality occurred during the four week study.

CLINICAL SIGNS

Sialorrhea following dose administration was seen in the 400 mg/kg male group starting on Day 14 and continuing until study termination. Sialorrhea was found in approximately 50% of the post-dose observations and all five of the high-dose males exhibited this clinical sign at least once in the last two weeks of the study. Discoloration of the hair of face and an unkempt hair coat in the inguinal region were seen in one 400 mg/kg male (Rat #369) on Day 29 of the study. Porphyrin tears were seen on Day 1 in Rat #360, a male from the 25 mg/kg dose group. Both of these observations were not considered treatment-related as they occurred only once and are often seen in control animals. An umbilical hernia was found in one male (Rat #352) from the control group on Day 8. This hernia appeared to have no adverse effect on the animal, and did not affect the outcome of the study.

One 400 mg/kg female (Rat #387) was depressed following administration of the test material on Days 2 and 3. This animal appeared to have had weight loss on Day 4, but subsequently appeared normal. Sialorrhea following dose administration was seen in the 400 mg/kg female group starting on Day 14 and continuing until study termination. Sialorrhea was found in approximately 50% of the post-dose observations and all five of the high-dose females exhibited this clinical sign at least once in the last two weeks of the study. In the 400 mg/kg female group, single rats were observed to have crusts or scaling and alopecia on the face (Rat 389) or alopecia on the back (Rat 386). Such lesions often result from abrasion on cage surfaces or interaction with cagemates and were not considered to be treatment-related.

BODY WEIGHT AND FEED CONSUMPTION

Slightly lower mean body weights were seen during the second week of dosing for all male groups given the test article. The differences in body weight on Day 28 amounted to 1.9%, 4.5%, and 6.1% less than the control mean weight for the low-, mid-, and high-dose groups, respectively. However, these differences were not statistically significant. Body weight growth for the 400 mg/kg female group was retarded between Days 0 and 4, but subsequently recovered, and at termination the mean body weight for this group was just 2.3% less than the control group; this difference was not significant. The low- and mid-dose female groups weighed slightly more than the control group. Mean feed consumption by groups given the test article was not different from the control groups.

HEMATOLOGY

No treatment-related hematology differences were noted for male groups at any dose level. Females from the 400 mg/kg dose group had significantly lower mean red blood cell count, hemoglobin concentration, and hematocrit when compared to the control group. No treatment-related blood cell morphology differences were noted for any dose group. All red blood cell indices were within normal limits indicating the presence of a minimal normochromic, normocytic anemia in the 400 mg/kg female group. Since no evidence of increased red blood cell destruction or turnover was found in other hematology tests, or during necropsy or histopathology examinations, the test article may have had an effect on erythropoiesis, rather than a direct toxic effect on circulating red blood cells.

CLINICAL CHEMISTRY

Both male and female rats from the 400 mg/kg group had significantly higher mean serum creatinine values when compared to their respective control groups. The differences were 16% for males and 13% for females. Mean serum glucose was statistically lower for all male groups treated with the test material when compared to the control value. The apparent decrease in serum glucose values in the treated male rats was due to extremely high serum glucose values in 2 out of the 5 control males. The serum glucose values from treated male rats were not significantly different from historical controls. For the 400 mg/kg male group, there were also significantly higher mean values for serum aspartate aminotransferase (AST) and sorbitol dehydrogenase (SDH) when compared to the control value. The mean AST value for the 400 mg/kg males was 31% higher than the control value. The SDH values for the 400 mg/kg group were variable; individual determinations for four of five animals were higher than those from animals in the other dose levels, and for two animals, they were nearly five-fold higher than the mean value for the control group. There were no statistical differences among female groups for mean glucose, AST or SDH values, although one 400 mg/kg female had an SDH value which was approximately 3.8-fold higher than the mean value for the female control group. No other treatment-related clinical chemistry differences were noted for any dose group of either sex.

ORGAN WEIGHTS

The mean absolute kidney weights for male groups administered the test material were slightly heavier than the mean kidney weight for the control group, though the differences were not dose-dependent. A statistical difference was seen only for the 25 mg/kg group. Relative (to body weight) kidney weights were statistically heavier for all male groups treated with the test material than for the control, but the differences did not follow a dose-related trend. The statistical differences were due to a combination of slightly heavier kidneys and slightly lower mean terminal body weights in all test article-treated male groups. No other differences in organ weights were seen for male rats.

No differences were seen in mean absolute or relative kidney weights for female rats. The mean absolute liver weight was slightly heavier for the 400 mg/kg group than for any other female group, but the difference was not statistically significant. The mean relative liver weight was significantly heavier for the 400 mg/kg female group than for the control group. There were no other significant differences in absolute or relative liver weights or any other differences in organ weights for female groups.

GROSS PATHOLOGY

No treatment-related observations were noted on gross pathology examination for any dose level of either sex.

HISTOPATHOLOGY

In the 400 mg/kg male and female animals, lesions which may be treatment-related included an increased severity of chronic focal inflammation of the liver (3/5 males, 2/5 females), and degeneration of the proximal convoluted tubules of the kidneys (2/5 males, 2/5 females). Similar liver lesions were observed in the control animals, but they were more severe and more widely distributed in the 400 mg/kg dose groups. The kidney lesions for both sexes involved only a few proximal convoluted tubules in each affected animal. No other abnormalities in histology were noted for any dose group of either sex.

DISCUSSION AND INTERPRETATION

In the probe study, doses of 800 mg/kg/day had a narcotic effect on the animals which resulted primarily in transient reduced activity levels and ataxia (females) after dose administration. At this dose level, one of four animals became moribund and was euthanatized. Thus narcosis at 800 mg/kg became a limiting factor in setting dose levels for the subsequent subacute study. Doses of 400 mg/kg/day also resulted in decreased activity in 1 of 2 female rats on the second and third day of dosing. No abnormal clinical signs were noted in the other 400 mg/kg animals or in the 200 mg/kg dose group. Based on the effects observed during the five-day probe study, dose levels of 0, 25, 100, and 400 mg/kg/day were chosen for the four-week study.

Administration of 21 doses of 4-methylcyclohexane methanol in corn oil to rats over a period of four weeks, at dose levels of 25, 100, or 400 mg/kg/day, did not result in mortality.

Sialorrhea following dose administration was noted in both the male and female 400 mg/kg groups from the second week to the end of the study. This condition is not uncommon with gavage studies when a chemical has an odor or a strong smell. One female rat in the 400 mg/kg dose group was depressed for two days during the first week of treatment, but appeared normal thereafter. Other clinical signs were considered incidental to treatment with the test article. Overall, the animals tolerated repeated dosing with the test article well and reduced activity levels which were a prominent clinical effect in the probe study were not prominent in the subacute study.

All male groups given the test material had mean body weights which were slightly lower than the control group. While the weight differences appeared to be dose-dependent, they were not statistically significant and were within the variability levels seen for normal growing rats. Females from the 400 mg/kg group initially lost weight, but recovered within the first week and the other female groups actually weighed slightly more than the control group. This information taken together suggests that the test article may have had a slight effect on the growth of male and female rats at the 400 mg/kg dose level but at lower dose levels, a consistent pattern was not present. Feed consumption was not affected by administration of any of the dose levels.

Hematology tests revealed a decreased mean red blood cell count, and lower hemoglobin and hematocrit levels for the female 400 mg/kg dose group. There were no other hematologic differences noted for any of the other groups. In the absence of any indicators of increased red blood cell destruction or turnover, the presence of a normochromic, normocytic anemia in the 400 mg/kg female group suggests that the test article may have interfered with the erythropoietic process, rather than having a direct effect on circulating red blood cells.

The kidney appeared to be slightly affected by administration of the test material. In the male rats, there were minor but statistically significant higher mean relative kidney weights for all treatment groups, and a significantly higher absolute kidney weight for the low-dose group. Interpretation of these results is difficult because the actual weight differences were small and did not follow a dose-related pattern. The heaviest mean kidney weights, both absolute and relative, were in the low-dose group. The females did not show any significant differences in kidney weights. Mean serum creatinine levels for both males and females at the 400 mg/kg dose level were statistically greater that the control levels. The differences were not clinically significant since the serum urea nitrogen levels were not affected in a similar manner. The highest serum creatinine levels correlated with the kidney lesions observed in the 400 mg/kg male and female rats. If the differences are real, an alternative interpretation of the data is that the test article interfered directly with the clinical chemistry assay for creatinine. Data to address this question directly are not available. Histopathological examination of the kidneys from the 400 mg/kg animals of both sexes revealed the presence of a minimal level of degeneration of the proximal convoluted tubules in 2 of 5 animals of each sex. The pathology data, while not overwhelming, suggest that at the high-dose level the test article had an effect on renal morphology. Functional correlates of the morphologic changes were not readily apparent in spite of the changes in clinical chemistry addressed above.

The liver also appeared to be slightly affected by administration of the test article. Effects on the liver consisted of significant increases in serum aspartate aminotransferase (AST) and sorbitol dehydrogenase (SDH) for the 400 mg/kg male group when compared to the control group. SDH was also higher in one of five female rats at the same dose level. The mean relative liver weight for the 400 mg/kg female group was significantly heavier than for the control group, but absolute liver weights for males and females and relative liver weights in males were not significantly altered by treatment with the test material. Microscopic examination of livers from the 400 mg/kg animals of both sexes revealed chronic focal liver inflammation of minimally increased severity and wider distribution than that seen in the control groups in five (three males, two females) of ten animals. The liver lesions in the 400 mg/kg male rats correlated with the increases in serum SDH but not with the differences in AST levels. The highest SDH levels for the 400 mg/kg females also correlated with the liver lesions.

In summary, administration of the test material for four weeks was associated with relatively minor changes in the erythropoietic system, the kidneys, and the liver at the 400 mg/kg/day dose level. None of the effects were indicative of more than minor toxicity, and all were most likely reversible. The no-observed-effect dose level for this study was 100 mg/kg/day.

TABLES

Tables 1 and 2 summarize the results of this study. Tables, including means and individual data points for body weight, feed consumption, hematology, clinical chemistries, and organ weights, and reports of individual contributors are available as appendices on request.

SIGNATURE PAGE

REPORT AUTHOR	DATE March 28, 1990
STUDY DIRECTOR	DATE April 3, 1990
UNIT DIRECTOR, MAMMALIAN TOXICOLOGY SECTION	DATE April 2, 1990
DIRECTOR, TOXICOLOGICAL SCIENCES LABORATORY	DATE Apr. 3, 1990
QUALITY ASSURANCE UNIT	DATE 3/28/90

SUMMARY OF REPEATED EXPOSURE STUDY (TABLE 1)

No. rats/group 5, No. of Treatments 21, Days of Experiment 29

Route of exposure: Gavage (mg/kg) Carrier: Corn Oil

Strain: CD®(SD)BR Sex: Male

Exposure concentration:	25	100	400
Weight gain	N*	N*	N*
Feed intake	N	N	N
Daily dose (mg/kg/day)	25	100	400
Clinical signs	*	N	*
Hematology:			
WBC	N	N	N
RBC	N	N	N
Hgb	N	N	N
Hct	N	N	N
MCV	N	N	N
MCH	N	N	N
MCHC	N	N	N
Platelets	N	N	N
Differential	N	N	N

Blood Cell Morphology: No treatment-related abnormalities were observed.

Clinical Chemistry:			
AST (GOT)	N	N	↑ 1
ALT (GPT)	N	N	N
AP	N	N	N
Urea Nitrogen	N	N	N
Glucose	N*	N*	N*
Creatinine	N	N	↑ 1
SDH	N	N	↑ 2
Organ weight:			
Kidneys			
Abs.	N*	N	N
Rel.	↑ 1	↑ 1	↑ 1
Liver			
Abs.	N	N	N
Rel.	N	N	N
Thymus			
Abs.	N	N	N
Rel.	N	N	N
Spleen			
Abs.	N	N	N
Rel.	N	N	N
Adrenal Glands			
Abs.	N	N	N
Rel.	N	N	N
Thyroid Glands			
Abs.	N	N	N
Rel.	N	N	N
Testes			
Abs.	N	N	N
Rel.	N	N	N

Gross pathology: No treatment-related abnormalities were observed.
Histopathology: Treatment-related effects were observed in the liver and kidneys.
Site of toxic action: Liver and Kidneys

Legend

↑ Increased ↓ Decreased 1 Slight 2 Moderate 3 Great N Normal ND Not Done
* See Text

261930L
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SUMMARY OF REPEATED EXPOSURE STUDY (TABLE 2)

No. rats/group 5, No. of Treatments 21, Days of Experiment 29

Route of exposure: Gavage (mg/kg) Carrier: Corn Oil

Strain: CD®(SD)BR Sex: Female

Exposure concentration:	25	100	400
Weight gain	N	N	N*
Feed intake	N	N	N
Daily dose (mg/kg/day)	25	100	400
Clinical signs	N	N	*
Hematology:			
WBC	N	N	N
RBC	N	N	↓ 1
Hgb.	N	N	↓ 1
Hct.	N	N	↓ 1
MCV	N	N	N
MCH	N	N	N
MCHC	N	N	N
Platelets	N	N	N
Differential	N	N	N
Blood Cell Morphology: No treatment-related abnormalities were observed.			
Clinical Chemistry:			
AST (GOT)	N	N	N
ALT (GPT)	N	N	N
AP	N	N	N
Urea Nitrogen	N	N	N
Glucose	N	N	N
Creatinine	N	N	↑ 1
SDH	N	N	N
Organ weight:			
Kidneys Abs.	N	N	N
Kidneys Rel.	N	N	N
Liver Abs.	N	N	N
Liver Rel.	N	N	↑ 1
Thymus Abs.	N	N	N
Thymus Rel.	N	N	N
Spleen Abs.	N	N	N
Spleen Rel.	N	N	N
Adrenal Glands Abs.	N	N	N
Adrenal Glands Rel.	N	N	N
Thyroid Glands Abs.	N	N	N
Thyroid Glands Rel.	N	N	N

Gross pathology: No treatment-related abnormalities were observed.
Histopathology: Treatment-related effects were observed in the liver and kidneys.
Site of toxic action: Erythropoiesis, Liver, and Kidneys

Legend

↑ Increased ↓ Decreased 1 Slight 2 Moderate 3 Great N Normal ND Not Done
* See Text

261986H
TX-90-5

ACUTE TOXICITY OF
90% min. 4-METHYLCYCLOHEXANE METHANOL

SYNONYM: MCHM

HAEL NO. 89-0081 ACC. NO. 907670

BY KENNETH P. SHEPARD, B.S.

TOXICOLOGICAL SCIENCES LABORATORY
HEALTH AND ENVIRONMENT LABORATORIES
EASTMAN KODAK COMPANY
ROCHESTER, NY 14652-3615

DATE OF STUDY COMPLETION JANUARY 26, 1990

261986H
TX-90-5

SUMMARY OF ACUTE TOXICITY STUDIES

Chemical: 4-Methylcyclohexane methanol
Synonym: MCHM

Accession No.: 907670

HAEL No.: 89-0081

Source Reference I.D. No.: X20511-3-3

Source: Eastman Kodak Company

Date of First Study Initiation: July 24, 1989

Comments: All animals were identified by metal ear tags/cage number. All specimens, raw data, and the final report of this work are stored in the archives of the Health and Environment Laboratories. Only limited analyses have been completed on the strength, purity, composition, stability, uniformity, and concentration of the test material. Professionals involved in this study other than the study director included: Gordon J. Hankinson, D.V.M., M.S., Laboratory Animal Medicine and Milan S. Vlaovic, D.V.M., Ph.D., Pathologist. Deviations from approved protocols or standard operating procedures included: None.

Director, Mammalian Toxicology Section
Study Director

January 26, 1990
Date

Director, Toxicological Sciences Laboratory

January 26, 1990
Date

ACUTE ORAL TOXICITY IN THE RAT

Dose levels tested: 625, 1250, and 2500 mg/kg

No. Rats/dose: 5 of each sex/dose

Vehicle: None

Solution/suspension: Administered as received

Initial Body weight range (g): Males: 204 - 245 Females: 153 - 188

Strain: Crl:CD®(SD)BR

Method of calculation: Weil method

Test SOP No.: TA 110, TA 120

DOSE mg/kg	NO. RATS DOSED(M,F)	NO. DEATHS (M,F)	TIME OF DEATH	WEIGHT GAIN* 1 WEEK(M,F)	WEIGHT GAIN* 2 WEEK(M,F)
625	5,5	0,0	----------	5+,5+	5+,5+
1250	5,5	0,5	Day 1 to Day 3	5+,--	5+,--
5000	5,5	5,5	Day 1	--,--	--,--

* + = Number of animals gaining weight
- = Number of animals losing weight

SUMMARY OF CLINICAL SIGNS		
DOSE (mg/kg)	CLINICAL SIGN	NO. OF RATS AFFECTED
625	Slight Weakness Progressing to Moderate Weakness	5M,5F
	Vasodilatation	5M,5F
	Brown Urine	1F
	Clinically Recovered	5M,5F
1250	Slight Weakness	5M
	Moderate Weakness	5M,5F
	Severe Weakness	5M,5F
	Vasodilatation	5M,5F
	Brown Urine	1F
	Decreased Feces	1F
	Hypothermia	1F
	Prostration	1F
	Clinically Recovered	5M
	Death	5F
2500	Severe Weakness	5M,5F
	Prostration	5M,5F
	Vasodilatation	5M,5F
	Death	5M,5F

ACUTE ORAL TOXICITY IN THE RAT CONT.

ESTIMATED ORAL LD_{50} IN RATS

MALES: 1768 mg/kg - 95% C.I. 1340 - 2333 mg/kg
FEMALES: 884 mg/kg - 95% C.I. 670 - 1166 mg/kg

REMARKS: The test article was slightly toxic by the oral route. For the selection of dose levels, a range finding study was conducted utilizing one animal of each sex per dose level, with body weights ranging between 154-214 grams. Dose levels of 5000, 2500, 1250, 625, and 312 mg/kg were used in the range finding study, with the test material administered as received. All animals given a dose of 5000 or 2500 mg/kg, and the female at the 1250 mg/kg dose level were found dead on the day following administration of the test material. No other deaths occurred over a 14-day observation period. Based on the range finding study, dose levels of 625, 1250, and 2500 mg/kg were chosen for the oral toxicity study.

In the acute oral toxicity study, abnormal clinical signs at a dose level of 2500 mg/kg included severe weakness, vasodilatation, and prostration in all animals on the day of administration of the test material. All females and two of five males died before examination on the day following dosing. Abnormal clinical observations in the remaining three males, which died later that day, included severe weakness, vasodilatation, and prostration.

At the 1250 mg/kg dose level, three of five females died on the day following administration of the test material and by Day 2 of the study, an additional female died. The remaining female died before clinical examinations on Day 3 of the study. Abnormal clinical signs on the day of dosing included moderate weakness which progressed to severe weakness, and vasodilatation in all animals. On the day following administration of the test material, only slight weakness was noted in males. In the two surviving females, abnormal clinical signs included severe weakness (2/2), prostration (1/2), and brown urine (1/2). By Day 2 of the study, all males appeared clinically normal and an additional female had died. Abnormal clinical signs noted in the remaining female included severe weakness, brown urine, decreased feces, and hypothermia. This female died the following day. No other abnormal clinical signs were noted, all males survived to scheduled necropsy, and all gained weight normally.

Abnormal clinical signs at the 625 mg/kg dose level were restricted to slight weakness which progressed to moderate weakness, and vasodilitation in all animals on the day of dosing. Brown urine was also noted from one female. On the day following administration of the test material, all animals appeared clinically normal. All animals at this dose level gained weight normally and survived the 14-day observation period.

ACUTE ORAL TOXICITY IN THE RAT CONT.

REMARKS
CONT: In the 2500 mg/kg dose group, treatment-related changes observed at necropsy included edema of the glandular gastric mucosa (5/5 males, 5/5 females), congestion of the gastric serosa (4/5 males, 5/5 females), and excessive mucus accumulation in the lumen of the duodenum (5/5 males, 5/5 females).

At the 1250 mg/kg dose level, no treatment-related changes were noted at necropsy of the males. Treatment-related changes in females included edema of the glandular (4/5) and non-glandular (2/5) gastric mucosa, congestion of the gastric serosa (2/5), excessive mucus accumulation in lumen of the duodenum (3/5), and brown discoloration of the inguinal hair (1/5).

No treatment-related changes were observed at necropsy in males or females at the 625 mg/kg dose level.

All other lesions observed at necropsy were not considered treatment-related. No tissue was collected for microscopic examination.

The test article was a gastrointestinal irritant as evidenced by edema of the gastric mucosa, congestion of the gastric serosa, and excessive accumulation of mucus in lumen of the duodenum. The cause of death for rats dying after exposure to the test material was not determined.

ACUTE DERMAL TOXICITY IN RATS

Dose levels tested: 2, 6, and 20 mL/kg

No. Rats/dose: 5 of each sex/dose

Vehicle: None

Preparation: Administered as received

Initial Body weight range (g): 2 and 6 mL/kg = (M) 176 - 196 (F) 160 - 170

20 mL/kg = (M) 271 - 296 (F) 196 - 205

Strain: Crl:CD®(SD)BR

Method of calculation: Weil method

Test SOP No.: TA 310

DOSE mL/kg	NO. RATS DOSED(M,F)	NO. DEATHS (M,F)	TIME OF DEATH	WEIGHT GAIN* 1 WEEK(M,F)	WEIGHT GAIN* 2 WEEK(M,F)
2	5,5	0,0	----------	5+,5+	5+,5+
6	5,5	5,5	Day 1 - Day 2	--,--	--,--
20	5,5	5,5	Day 1	--,--	--,--

* + = Number of animals gaining weight
- = Number of animals losing weight

SUMMARY OF CLINICAL SIGNS		
DOSE (mL/kg)	CLINICAL SIGN	NO. OF RATS AFFECTED
2	Slight Weakness	5M,5F
	Signs at the Application Site	
	- Slight Erythema	5M,5F
	- Necrosis	5M,5F
	- Escars	5M,5F
	- Scarring	5M,4F
	Clinically Recovered	5M,3F
6	Slight Weakness	5M,5F
	Death (Day 1-before clinical observations)	2M,5F
	Severe Weakness	3M
	Prostration	3M
	Signs at the Application Site	
	- Slight Erythema	3M
	- Necrosis	3M
	Death (Day 1-after clinical observations)	1M
	Death (Day 2)	2M

(Continued)

ACUTE DERMAL TOXICITY IN RATS CONT.

SUMMARY OF CLINICAL SIGNS CONT.		
DOSE (mL/kg)	CLINICAL SIGN	NO. OF RATS AFFECTED
20	Slight Weakness Progressing to Severe Weakness	5M,5F
	Prostration	5M,5F
	Vasodilatation	5M,5F
	Death (Day 1)	5M,5F

ESTIMATED DERMAL LD_{50} IN RATS

MALES: 3.6 mL/kg - 95% C.I. 2.2 - 5.6 mL/kg
FEMALES: 3.6 mL/kg - 95% C.I. 2.2 - 5.6 mL/kg

SKIN ABSORPTION: Evident
(Based on Dermal Toxicity study)

REMARKS: The test article was moderately toxic by the dermal route. All doses were applied to the skin after the hair had been removed with an electric clipper. An occlusive wrap was used to hold the test material against the skin for 24 hours, and at the end of exposure, residual test material was washed off with running water.

At the 20 mL/kg dose level, slight weakness was observed in all animals one hour after the start of exposure. By four hours, all animals had clinical signs of severe weakness, prostration, and vasodilatation. All animals died before termination of the 24-hour exposure period.

For the selection of additional dose levels, a range finding study was conducted at dose levels of 2.5, 5, and 10 mL/kg. One male rat was used at each dose level, with body weights ranging between 183-206 grams. Only the animal at the 2.5 mL/kg dose level survived a 7-day period. Based on the range finding studies, additional dose levels of 2 and 6 mL/kg were selected for the dermal toxicity study.

At the 6 mL/kg dose level, slight weakness was noted in all animals during exposure to the test material, and two of five males and all females died before termination of the exposure period. Systemic toxicity observed in the remaining three males that survived the exposure period included severe weakness and prostration. Slight erythema and necrosis were observed at the site of application of the test material. One of three males

ACUTE DERMAL TOXICITY IN RATS CONT.

REMARKS CONT.: died one hour and thirty minutes after termination of the exposure period, and by the following day, the last two males had died.

At the 2 mL/kg dose level, all animals appeared normal during the exposure period. At the end of the exposure period, slight erythema and necrosis at the application site and slight weakness were noted for all animals. Necrosis was observed only at the end of exposure, while erythema persisted to Day 3 of the study. By Day 2 of the study, eschars at the application site were observed on all animals, and by Day 9, scarring was also noted at the application site on nine of ten animals. By termination of the 14-day observation period, animals had either clinically recovered or abnormal signs were limited to escars and scarring. All animals survived to scheduled necropsy, and all gained weight normally.

Treatment-related changes observed at necropsy consisted of focal necrosis of the skin of the back for all animals in the 20 mL/kg dose group, and for four of five males and all females in the 6 mL/kg dose group.

At the 2 mL/kg dose level, treatment-related changes observed at necropsy included an eschar (1/5 females) and scars (2/5 females) on the skin of the back. No treatment-related changes were observed in males.

All other lesions observed at necropsy were not considered treatment-related and no tissue was collected for microscopic evaluation. The cause of death for rats dying after exposure to the test material was not determined.

The test article was a skin irritant as evidenced by necrosis, eschars, and scars on the skin of the back. Percutaneous absorption was evident based on clinical signs of weakness, vasodilatation, and prostration during the exposure period.

ACUTE TOXICITY - DERMAL IRRITATION IN GUINEA PIGS

24 hr. Occluded Single Dose

Slight Moderate Strong Severe

Dose levels tested: 0.5 mL

No. guinea pigs/dose: 5

Vehicle: None

Preparation: Administered as received

Initial Body weight range (g): 414 - 443

Strain: Crl:(HA)BR Hartley

Test SOP No.: TA 160

Sex: Male

Acute Skin Irritation

Slight

Moderate

Strong

Estimated Corrosivity

No

Questionable

Yes

RESULTS

ANIMAL NO.	MAXIMUM EFFECT		WEIGHT CHANGE (g)	
	SKIN - 24/48 HOURS	SKIN - 2 WEEKS	1 WEEK	2 WEEKS
606	Mod. Ery., Slt. Ed., Mod. N., & Slt. Esc.	Slt. Esc.	+44	+94
607	Mod. Ery., Mod. Ed., Mod. N., & Mod. Esc.	Slt. Esc.	+48	+110
608	Mod. Ery., Slt. Ed., Mod. N., & Slt. Esc.	Slt. Esc.	+45	+94
609	Mod. Ery., Slt. Ed., Mod. N., & Mod. Esc.	Normal	+50	+85
610	Mod. Ery., Mod. Ed., Mod. N., & Mod. Esc.	Slt. Esc.	+40	+75

KEY: Slt. = Slight Ery. = Erythema Esc. = Eschar St. = Staining
Mod. = Moderate Ed. = Edema Scr. = Scarring Ero. = Erosion
Str. = Strong N. = Necrosis V. Slt = Very Slight

REMARKS: The test article was a strong skin irritant. A dose of 0.5 mL was applied to each depilated guinea pig abdomen, and an occlusive wrap was used to hold the test material against the skin for 24 hours. During the first 48 hours after removal of the occlusive wrap,

ACUTE TOXICITY - DERMAL IRRITATION IN GUINEA PIGS CONT.

REMARKS
CONT: signs of irritation included moderate erythema (5/5), slight (3/5) to moderate (2/5) edema, moderate necrosis (5/5), and slight (2/5) to moderate (3/5) eschar formation at the site of application of the test material. By 72 hours, the two animals with slight eschar formation developed moderate eschars. On Day 7, signs of irritation included slight erythema (1/5) and slight (1/5) to moderate (4/5) eschars. By Day 14, a single animal appeared normal, and slight eschars at the application site persisted in the remaining four animals. All animals survived the 14-day observation period, and all gained weight normally. Based on the absence of clinical signs of toxicity or reduced weight gain, percutaneous absorption was not evident under the conditions of this study.

ACUTE TOXICITY - REPEATED SKIN

Dose levels tested: 0.5 mL drop-on

No. of doses: 9 No. of days: 11

No. guinea pigs/dose: 5

Vehicle: None

Preparation: Administered as received

Initial body weight range (g): 401 - 432

End of study body weight range (g): 429 - 496

Strain: Crl:(HA)BR Hartley

Test SOP No.: TA 170

Sex: Not Determined

RESULTS	GREATEST EFFECT				
	ERYTHEMA	EDEMA	NECROSIS	ESCHAR	OTHER
1ST DOSE	0	0	0	0	0
LAST DOSE	2	2	2	3	0

REMARKS: There was exacerbation of the irritant response with repeated application of the test material. The test material was applied topically, as received, to the backs of guinea pigs after the hair had been removed with an electric clipper. After a single dose, no signs of irritation were noted. At termination of the first week of the study, signs of irritation were limited to slight erythema at the site of application on all animals. After the seventh application over a period of nine days, signs of irritation included erythema, edema, necrosis, and eschar formation. After nine daily applications over a period of eleven days, signs of irritation consisted of moderate erythema (5/5), moderate edema (5/5), moderate necrosis (5/5), and moderate (2/5) to strong (3/5) eschars. Based on the absence of clinical signs of toxicity or reduced weight gain, percutaneous absorption was not evident under the conditions of this study.

ACUTE TOXICITY - SKIN SENSITIZATION
KODAK FOOTPAD METHOD

No. guinea pigs per dose: 10

Irritation drop-on body weight range (g): 417 - 471

Induction body weight range (g): 345 - 471

Strain: Crl:(HA)BR Hartley

Test SOP No.: TA 180

Sex: Male

Estimated Human Risk
Low
Moderate
High

Irritation Drop-on

Concentrations tested: 0.05 mL of compound in 5.0 mL of Acetone + Dioxane + Guinea Pig Fat.

Highest average score: 0

Induction and Challenge Study

Induction preparation: 0.05 mL of a 1.0% solution of compound in Freund's Complete Adjuvant.

No. of challenge doses: 1

Challenge preparation: 1.0 mL of compound in 10.0 mL of Acetone + Dioxane + Guinea Pig Fat.

SENSITIZATION	NUMBER POSITIVE				TOTAL SCORE
	NORMAL	SLIGHT	MODERATE	STRONG	
FREUND'S ONLY	10				0
1% CPD. IN FREUND'S	10				0

REMARKS: The test article has a low potential to cause human sensitization. No reaction was observed at challenge in any of the animals previously induced with Freund's adjuvant or the test article in Freund's adjuvant.

ACUTE TOXICITY - RABBIT EYE IRRITATION

Dose level tested: 0.1 mL
No. of doses: 1
No. rabbits/dose: 3 washed / 3 unwashed
Vehicle: None
Washing agent: Distilled water
Body weight range: Not determined
Strain: New Zealand white
Test SOP No.: TA 150
Sex: Not Determined

Acute Eye Irritation
Slight
Moderate
Strong

RESULTS	3 UNWASHED EYES					
	IMMED.	1 HR.	24 HR.	48 HR.	72 HR.	DAY 7
Initial	Slt.					
Conjunctiva		Mod.	Mod.	Mod.	Mod.	
Lids		Mod.	Mod.	Mod.	Slt.	
Nict. Membrane		Mod.	Mod.	Mod.	Mod.	
Corneal Opacity			Mod.			
Iris				Mod.		
Adnexal Stain			2/3			
Corneal Stain			3/3			
Discharge		Mod.				
Number Normal	0	0	0	0	0	3

RESULTS	3 WASHED EYES					
	IMMED.	1 HR.	24 HR.	48 HR.	72 HR.	DAY 7
Initial	Slt.					
Conjunctiva		Mod.	Slt.	Slt.		
Lids		Slt.				
Nict. Membrane		Mod.	Slt.	Slt.		
Corneal Opacity						
Iris						
Adnexal Stain			0/3			
Corneal Stain			0/3			
Discharge		Slt.				
Number Normal	1	0	0	0	3	3

KEY: Slt. = Slight Mod. = Moderate Str. = Strong

ACUTE TOXICITY - RABBIT EYE IRRITATION CONT.

NO. RESPONDING	Slight	Moderate	Strong	Severe	Fluorescein Stain	
					Adnexa	Cornea
UNWASHED EYES		3/3			2/3	3/3
WASHED EYES	3/3				0/3	0/3

REMARKS: The test article was a moderate eye irritant. In unwashed eyes, signs of irritation consisted of moderate erythema and edema of the lids, conjunctivae, and nictitating membranes in all eyes at the one-hour observation period. In addition, moderate discharges were noted from all unwashed eyes one hour after dosing. Slight corneal opacity was evident in unwashed eyes only at the 24-hour observation period. When unwashed eyes were tested with fluorescein dye 24 hours after dosing, there was corneal staining in all eyes and staining of the nictitating membranes in two of three eyes. Injection of the iris was noted in each unwashed eye at the 48-hour observation period. By Day 7 of the study, all unwashed eyes appeared clinically normal.

Immediate washing was palliative. In washed eyes, signs of irritation included moderate erythema (3/3) and slight edema (2/3) of the conjunctivae and nictitating membranes and slight erythema of the lids (3/3) at the one-hour observation period. A slight discharge from all washed eyes was noted one hour after dosing. No corneal opacity was noted in washed eyes at any time during the 7-day observation period. At 24 hours, no corneal or adnexal staining were observed when washed eyes were tested with fluorescein dye. By 72 hours, all washed eyes appeared normal.

Q.A. INSPECTION STATEMENT
(CFR 58.35(B)(7) 792.35(B)(7) 160.35(B)(7))

STUDY: 89-0081-1 STUDY DIRECTOR: TOPPING, D.C.
ACCESSION NUMBER: 907670

STUDY TYPE: ACUTE TOXICITY REPORT

M. A. James
(AUDITOR, QUALITY ASSURANCE UNIT)

1/25/90
DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT OF THE HAEL, EASTMAN KODAK COMPANY, ROCHESTER, N.Y. AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECTION DATES	STUDY/PHASE INSPECTED	STATUS REPORT DATES
07/24/89	ACUTE DERMAL IRRITATION TEST PROTOCOL APPENDIX SUBMISSION	
07/24/89	REPEATED SKIN IRRITATION PROTOCOL APPENDIX SUBMISSION	
07/26/89	SENSITIZATION PROTOCOL APPENDIX SUBMISSION IRRITATION DROP-ON	
07/31/89	RABBIT EYE IRRITATION PROTOCOL APPENDIX SUBMISSION	
08/01/89	ACUTE DERMAL TOXICITY (LD50) PROTOCOL APPENDIX SUBMISSION DOSE CALCULATIONS TEST ARTICLE APPLICATION TO TEST SYSTEM	
08/02/89	SENSITIZATION PROTOCOL APPENDIX SUBMISSION FOOT PAD INJECTION	
08/02/89	ACUTE ORAL LD50 PROTOCOL APPENDIX SUBMISSION	
08/07/89	ACUTE ORAL LD50 NECROPSY	
08/08/89	ACUTE DERMAL TOXICITY (LD50) PROTOCOL APPENDIX SUBMISSION TEST ARTICLE WEIGHT DOSE CALCULATIONS TEST ARTICLE APPLICATION TO TEST SYSTEM	
08/09/89	SENSITIZATION 1 WEEK CHALLENGE (TEST ARTICLE-CARRIER-MIXTURE DROP-ON)	08/09/89
01/25/90	ACUTE TOXICITY REPORT FINAL REPORT REVIEW	01/25/90